Environmental Health Perspectives
Vol. 23, pp. 233-239, 1978

Effect of Polybrominated Biphenyls on Hepatic Excretory Function in Rats and Mice

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The purpose of this investigation was to determine the influence of polybrominated biphenyls (PBBs) on hepatic excretory function in developing and adult rats and mice. Prenatal or postnatal dietary exposure to PBBs (50 ppm in diet of pregnant or lactating mother or in diet of rat weanlings) resulted in elevated liver weight in developing rats. In 15-day-old rats that had been treated with PBBs, increased liver weight correlated to enhanced ouabain transport from plasma into bile. Liver weight was also elevated in 21, 35, and 49-day-old rats exposed to PBBs, but this effect was not associated with stimulation of ouabain transport in these animals. However, adult rats fed 100 ppm PBBs for two weeks had significantly lower plasma concentrations of sulfobromophthalein (BSP) and increased biliary excretion of BSP, when compared to controls. PBBs-fed adult rats also excreted a greater percentage conjugated BSP (BSP-GSH) into bile. Two week dietary treatment of 100, 150, and 200 ppm PBBs resulted in enhanced initial disappearance of indocyanine green (ICG) from plasma of adult mice. However, dietary doses of 100 and 200 ppm PBBs to adult mice was not associated with enhanced capacity for ouabain excretion. In contrast, treatment with PBBs through the mother's diet (50 ppm) resulted in an almost twofold increase in cumulative ouabain excretion in 15-day-old mice. The results suggest that PBBs stimulate hepatic drug elimination in rats and mice, but the magnitude of the effect is dependent on age and transported compound.

Introduction

Polybrominated biphenyls (PBBs) are used commercially as flame retardants. The adverse consequences of exposure to PBBs are not completely known. However, a similar class of compounds, the polychlorinated biphenyls (PCBs), has been extensively studied, and PBBs may share many of the biological and toxic properties of PCBs. PCBs are known to cause chloracne (*1*) and Yusho disease in humans (*2*).

In laboratory animals one of the most prominent effects of both PBBs and PCBs is induction of a large increase in liver weight and, in addition, stimulation of hepatic drug-metabolizing capabilities (*3–6*). PBBs are several times more potent than PCBs in stimulating microsomal enzyme activity (*7*). PBBs and PCBs represent a class of hepatic mixed function oxidase stimulators which exhibit characteristics of both phenobarbital and 3-methylcholanthrene (*5, 6, 8, 9*), two agents which are distinct in their stimulating properties (*10, 11*). Microsomal enzyme stimulators such as phenobarbital may also enhance bile flow and the biliary excretion of drugs; 3-methylcholanthrene does not produce these effects (*12–14*). The purpose of this investigation was to determine the influence of PBBs on the liver as an organ for drug excretion.

Methods

Swiss-Webster mice (30 g) and adult male (200–250 g), timed pregnant or lactating female (with litters of 8–10 offspring) Sprague-Dawley rats were obtained from Spartan Research Animals Inc. (Haslett, Michigan). PBBs (FireMaster BP-6, Michigan Chemical Co.) were dissolved in diethyl ether or acetone (10 ml/kg diet) and were thoroughly mixed with powdered food pellets over a 10-min

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period. The control diet (0 ppm) was powdered food to which only the solvent had been added.

Hepatic transport of ouabain was determined in developing rats by quantitating the time dependent disposition of tritium following administration of [^{3}H]-ouabain. Developing rats (1.0 mg/kg ouabain) were administered ouabain via the tail vein and, at various times following ouabain administration, animals were anesthetized with ether, a blood sample was taken (with a heparinized syringe) by cardiac puncture, and the entire liver and small intestine were rapidly removed. The quantity of radioactivity in the intestine was measured to estimate biliary excretion of ouabain (*15*). Samples of liver, plasma, and intestinal homogenate were solubilized and when solubilization was complete, the samples were acidified by the addition of 0.5 ml 4.4*M* HNO_3 and counted in 12 ml of toluene–Triton X-100 (2:1) scintillation cocktail (*16*). In some experiments Dimilume counting solution (Packard Instrument Co.) was used. The effect of PBBs on ouabain excretion was determined similarly in developing and adult mice. Cumulative ouabain excretion was quantitated by measuring tritium in the intestine following a tail vein (0.1 mg/kg ouabain to adult mice) or intraperitoneal (1.0 mg/kg ouabain to 15-day-old mice) dose of [^{3}H]-ouabain. Radioactivity in all samples was determined with a Packard Model 3380 liquid scintillation spectrometer equipped with automatic external standard for quench correction (Packard Instrument Company, Downers Grove, Ill.).

The disappearance of indocyanine green (ICG) (Hynson, Westcott, and Dunning, Inc., Baltimore, Md.) from plasma was determined in adult mice and in 21-day-old rats following a single 40 mg/kg dose of the dye via the tail vein. At various times following administration of ICG, blood was obtained by cardiac puncture with a syringe rinsed in sodium oxalate. Concentration of ICG in plasma was determined by diluting the plasma sample with water and measuring absorbance of 805 nm (*17*).

Plasma disappearance and biliary excretion of sulfobromophthalein (BSP) (Hynson, Westcott, and Dunning, Inc.) was determined in adult rats following a single 120 mg/kg dose of BSP. Rats were anesthetized with 3 ml/kg of Equi-Thesin (Jensen-Salsbery, Inc., Kansas City, Mo.), and the femoral artery and vein were cannulated with PE_{50} polyethylene tubing and the bile duct cannulated with PE_{10} tubing. The animals were placed under a heat lamp to maintain body temperature. BSP was injected through the venous cannula and blood samples (0.3 ml) were drawn into a heparinized syringe through the arterial cannula at various time intervals. Bile was collected for 40 min in 10-min intervals. Plasma and bile samples were analyzed for BSP by measuring absorbance at 580 nm following appropriate dilution with 0.1*N* NaOH. Metabolites of BSP in bile were separated by paper chromatography (*18*) and quantitated by measuring absorbance at 580 nm following elution into distilled water.

Statistical evaluation of the data was made by Student's *t*-test (*19*). The level of significance was chosen as $p < 0.05$.

Results

Two week dietary exposure to as much as 200 ppm PBBs did not affect food consumption or body weight in adult mice (data not shown), however, a dose–dependent increase in liver weight was observed following PBBs (Table 1). Fifteen-day-old mice whose mothers were fed 50 ppm PBBs beginning at birth also had significantly elevated liver weight (Table 1). In developing rats, exposure to PBBs resulted in significant increases in liver weight to body weight ratios when compared to controls on postnatal days 15, 21, 35, and 49 (Table 1). In addition, when compared to 15-day-old rat neonates whose natural and foster mother received no dietary PBBs, a significant elevation of liver to body weight ratio was detected in 15-day-old rats exposed to PBBs prenatally, postnatally, and combined pre- and postnatally (Table 1).

The effect of PBBs on elimination of ouabain from plasma of 15, 21, 35, and 49-day-old rats is depicted in Figure 1. Exposure to PBBs began at birth by addition of 50 ppm PBBs to the mother's diet. Exposure was continued at postnatal day 28 through the weanling's diet. Plasma concentrations of ouabain were significantly lower than control values in PBB-exposed 15-day-old rats 3, 20, and 40 min following ouabain injection and in PBB-treated 21-day-old rats 3 min following ouabain injection. In contrast, plasma concentrations of ouabain in 35 and 49 day old PBB-exposed rats were not significantly different from plasma ouabain concentrations in the controls (Fig. 1). When compared to control rats of the same age, cumulative 40-min intestinal ouabain content, an estimate of biliary excretion of ouabain, was significantly elevated in PBB-exposed 15-day-old rats but not in 21, 35, and 49-day-old PBB-exposed animals (Table 2).

Following a single bolus ouabain injection, plasma concentrations of ouabain were significantly lower in 15-day-old rats exposed to PBBs prenatally (from day 8 of gestation to birth) and/or postnatally (from birth to postnatal day 15) when compared to 15-day-old controls (0-0) (Fig. 2). Among all of the dietary treatments, prenatal exposure to PBBs

Table 1. Effect of dietary PBBs on liver weight.

Species	Age, days	PBBs, ppm[a]	Duration	Liver weight/ body weight, %	% of respective control
Mouse	15	0	15 days (postnatal)	4.2 ± 0.1	
	15	50	15 days (postnatal)	8.4 ± 0.2[b]	200
	Adult	0	2 weeks	4.8 ± 0.1	
	Adult	50	2 weeks	6.8 ± 0.2[b]	142
	Adult	100	2 weeks	8.2 ± 0.2[b]	171
	Adult	150	2 weeks	8.9 ± 0.1[b]	185
	Adult	200	2 weeks	10.4 ± 0.1[b]	217
Rat	15	0	15 days (postnatal)	3.2 ± 0.1	
	15	50	15 days (postnatal)	4.6 ± 0.1[b]	144
	15	50	15 days (prenatal)	3.5 ± 0.1[b]	109
	15	50	30 days (pre+postnatal)	5.0 ± 0.1[b]	156
	21	0	21 days (postnatal)	4.0 ± 0.1	
	21	50	21 days (postnatal)	6.4 ± 0.1[b]	160
	35	0	35 days (postnatal)	4.0 ± 0.1	
	35	50	35 days (postnatal)	6.2 ± 0.2[b]	155
	49	0	49 days (postnatal)	4.7 ± 0.1	
	49	50	49 days (postnatal)	6.6 ± 0.1[b]	140

[a] Concentration of PBBs in diet (animals younger than 28 days, weaning age, received PBBs through mother's diet).
[b] Significantly different from 0 ppm PBBs ($p < 0.05$).

(50-0) was least effective in enhancing ouabain elimination from plasma. Similarly, cumulative 40-min intestinal ouabain content, an estimate of biliary excretion of ouabain, was highest in rats exposed to PBBs postnatally and was affected by prenatal PBB exposure to a lesser, but statistically significant, extent when compared to controls (Table 2). When compared to values from control (0-0) rats, 15-day-old animals exposed to PBBs postnatally (0-50), or pre- and postnatally (50-50) had significantly higher hepatic ouabain content 3 min following ouabain injection (Fig. 3). These increases reflect the effect of PBBs on liver weight (Table 1).

Similar results were observed in developing mice. Elevation in cumulative excretion of ouabain was observed in 15-day-old but not adult mice exposed to PBBs (Table 2).

In adult mice and 21-day-old rats, treatment with PBBs resulted in enhanced initial disappearance of ICG from plasma (Table 3 and Fig. 4). Plasma concentrations of ICG were significantly lower than control values 10 and 15 min following injection of ICG in mice fed 100 and 150 ppm PBBs. In mice fed 200 ppm PBBs, plasma concentration of ICG was significantly lower than control value 15 min following ICG injection (Fig. 4). The disappearance of ICG from plasma of 21-day-old control rats and rats exposed to PBBs is depicted in Table 3 by the rate of ICG elimination from plasma. The rate of ICG elimination from plasma was significantly greater in 21-day animals whose mothers were fed 50 ppm PBBs (Table 3).

Two week dietary exposure of adult rats to 100 ppm PBBs resulted in enhanced elimination of BSP from plasma and increased biliary excretion of BSP (Table 4). In addition, PBB-fed adult rats excreted a greater percentage conjugated BSP into bile (Table 4).

Table 2. Effect of polybrominated biphenyls (PBBs) on cumulative hepatic excretion of ouabain.[a]

Species	Age, days	Dietary PBBs, ppm	Duration	Ouabain excretion, % of dose
Rat	15	0[b]	15 days (postnatal)	12.2 ± 1.1
	15	50[b]	15 days (postnatal)	38.9 ± 1.6[d]
	15	50- 0[c]	15 days (postnatal)	17.4 ± 1.4[d]
	15	0-50[c]	15 days (postnatal)	27.4 ± 1.1[d]
	15	50-50[c]	30 days (pre+postnatal)	27.7 ± 2.8[d]
	21	0[b]	21 days (postnatal)	17.8 ± 0.6
	21	50[b]	21 days (postnatal)	20.2 ± 2.6
	35	0[b]	35 days (postnatal)	21.1 ± 5.4
	35	50[b]	35 days (postnatal)	20.6 ± 3.2
	49	0[b]	49 days (postnatal)	34.3 ± 1.5
	49	50[b]	49 days (postnatal)	39.8 ± 1.9
Mouse	15	0[b]	15 days (postnatal)[b]	24.7 ± 3.6
	15	50[b]	15 days (postnatal)[b]	46.3 ± 1.3[d]
	Adult	0[b]	2 weeks[b]	30.7 ± 3.4
	Adult	100[b]	2 weeks[b]	35.9 ± 2.5
	Adult	200[b]	2 weeks[b]	39.1 ± 2.0

[a] [^{3}H]-Ouabain was injected via the tail vein (0.1 mg/kg to adult mice or 1.0 mg/kg to developing rats) or intraperitoneally (1.0 mg/kg to 15-day-old mice) and after 40 min (in developing rats), 60 min (in adult mice) or 120 min (in 15-day-old mice), intestine was analyzed for tritium.

[b] Concentration of PBBs in diet (animals younger than 28 days, weaning age, received PBBs through mother's diet).

[c] Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50); 50 ppm prenatal, 50 ppm postnatal (50-50).

[d] Significantly different from 0 ppm PBBs at corresponding age ($p < 0.05$).

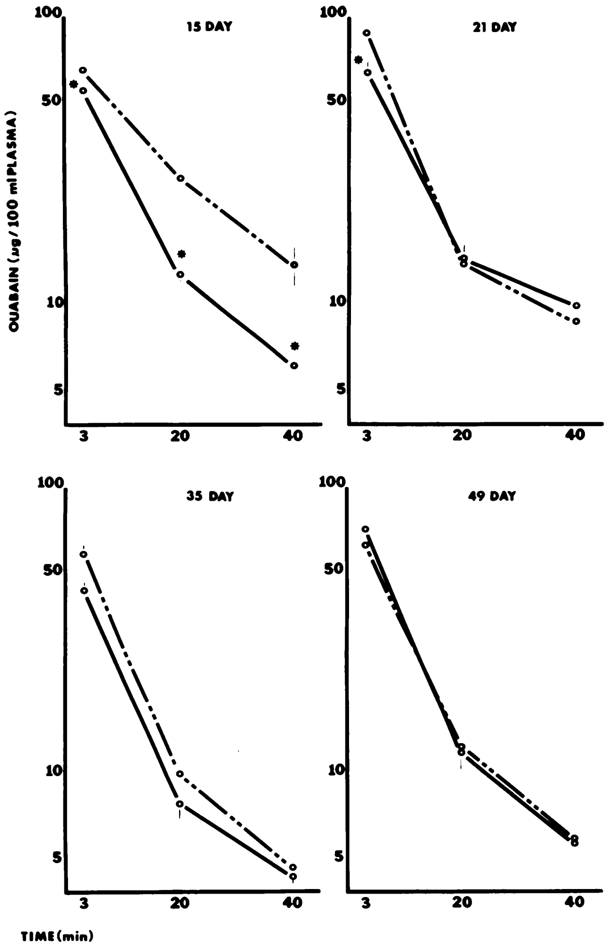

FIGURE 1. Effect of exposure to polybrominated biphenyls (PBBs) on elimination of [^{3}H]-ouabain from plasma of rats of ages ranging from 15 to 49 days: (--) 0 ppm PBBs; (—) 50 ppm PBBs. Young rats were exposed to PBBs by placement of 50 ppm PBBs in diet of lactating mother. At weaning, postnatal day 28, rats were weaned onto the same diet fed to mother. Rats were administered [^{3}H]-ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 min, blood was obtained by cardiac puncture and plasma samples analyzed for tritium (ouabain). Each point represents the mean ± S.E. for four rats obtained from four litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. The asterisks (*) indicates plasma ouabain concentrations significantly different from values obtained from rats exposed to 0 ppm PBBs ($p < 0.05$).

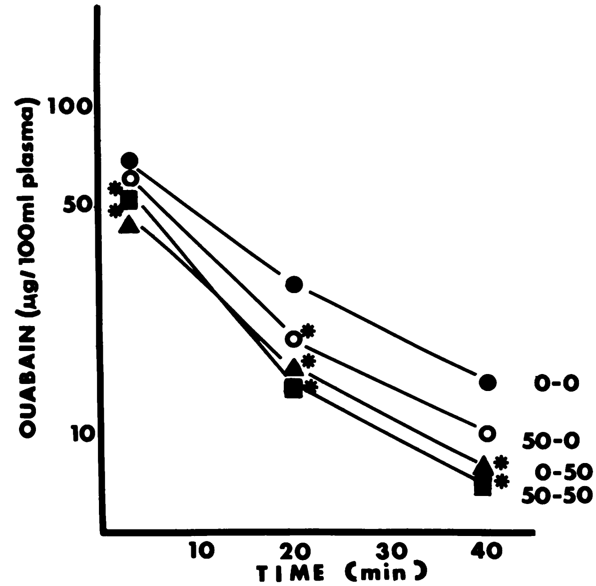

FIGURE 2. Effect of pre- and/or postnatal exposure to polybrominated biphenyls (PBBs) on elimination of [^{3}H]-ouabain from plasma of 15-day-old rats. Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: (0-0) 0 ppm prenatal, 0 ppm postnatal; (50-0) 50 ppm prenatal, 0 ppm postnatal; (0-50) 0 ppm prenatal, 50 postnatal (50-50), 50 ppm prenatal, 50 ppm postnatal. Rats were administered [^{3}H]-ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 min, blood was obtained by cardiac puncture and plasma samples analyzed for tritium (ouabain). Each point represents the mean for six to eight rats obtained from four litters. Standard error (not shown for clarity) was approximately 10% of mean value. The asterisks (*) indicates plasma ouabain concentration significantly different from values obtained from 0-0 ($p < 0.05$).

Discussion

It has been demonstrated that PBBs are potent stimulators of hepatic drug metabolism (5–7). The results of this investigation demonstrate that treatment with PBBs may also result in stimulation of hepatic excretory function.

Prenatal and/or postnatal exposure to PBBs resulted in stimulation of hepatic excretory function in 15-day-old rats (Fig. 1 and Table 2) and 15-day-old mice (Table 2). This was demonstrated by enhanced rate of elimination of ouabain from plasma (Figs. 1 and 2) and increased cumulative intestinal ouabain content (Table 2) in 15-day-old treated animals when compared to 15-day-old controls.

The effect of PBBs on ouabain transport in 15-day-old rats appeared to parallel the effect on liver mass. Stimulation of ouabain transport was most evident in rats receiving postnatal exposure to PBBs, and this effect coincided with the greater elevation in liver weight in these animals when compared to rats receiving prenatal (50-0) exposure (Figs. 1 and 2 and Table 1). However, the large increase in liver mass in 21, 35, and 49-day-old rats exposed to PBBs (Table 1) was not associated with enhanced elimination of ouabain from plasma or increased ouabain excretion (Fig. 1 and Table 2). Moreover, two week dietary exposure to 100 or 200 ppm PBBs to adult mice resulted in a marked increase in liver mass (Table 1) but no effect on the ability to excrete ouabain (Table 2). Thus, two fac-

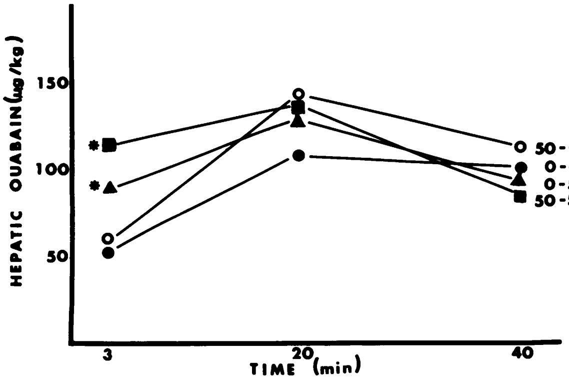

FIGURE 3. Effect of pre- and/or postnatal exposure to polybrominated biphenyls (PBBs) on hepatic content of [^{3}H]-ouabain in 15-day-old rats. Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: (0-0) 0 ppm prenatal, 0 ppm postnatal; (50-0) 50 ppm prenatal, 0 ppm postnatal; (0-50) 0 ppm prenatal, 50 ppm postnatal; (50-50) 50 ppm prenatal, 50 ppm prenatal. Rats were administered [^{3}H]-ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 min, samples of liver were analyzed for tritium (ouabain). Each point represents the mean for six to eight rats obtained from four litters. Standard error (not shown for clarity) was approximately 10% of mean value. The asterisks (*) indicates hepatic content of ouabain significantly different from values obtained from 0-0 ($p < 0.05$).

tors that may be important in stimulation of drug transport following PBBs are liver mass and age.

Elimination of xenobiotics from plasma into bile occurs in a stepwise manner. Drug transport into bile requires specific uptake into liver, intrahepatic metabolism and storage, and finally secretion from liver into bile. Ouabain is not metabolized in the liver prior to biliary excretion in rats (*21*) or mice (*22*); however, treatment with PBBs resulted in a significant increase in hepatic ouabain content (μg/kg body weight) 3 min following ouabain administration in 15-day-old rats (Fig. 3). The increase in hepatic ouabain content was due, to some extent, to increased ouabain concentration (data not shown) but the magnitude of this effect mainly reflected the effect of PBBs on liver weight (Table 1). Nonetheless, these data suggest that stimulation of ouabain transport in 15-day-old rats may be attributed to enhanced ouabain uptake into liver.

The mechanisms for hepatic uptake may also be stimulated following PBBs in the older rats. The initial rate of removal of indocyanine green (ICG) from plasma was enhanced following exposure to PBBs in 21-day-old rats (Table 3). Since the initial rate of elimination of drugs from plasma represents hepatic uptake (*23*), it is likely that treatment with PBBs resulted in enhanced uptake capacity in these animals. Following PBBs, stimulation of hepatic

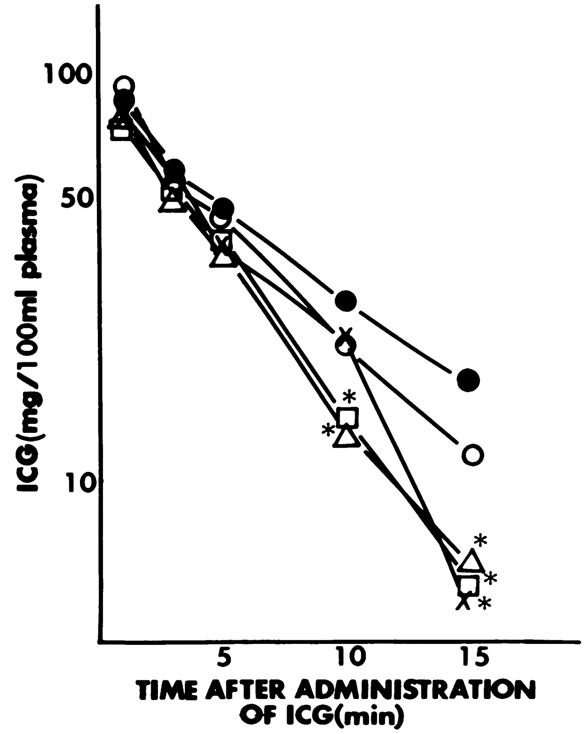

FIGURE 4. Disappearance of indocyanine green (ICG) from plasma of mice fed various doses of polybrominated biphenyls (PBBS) for 2 weeks prior to administration of ICG (40 mg/kg, iv). (•) 0 PBBs; (o) 50 ppm PBBs; (△) 100 ppm PBB; (□) 150 ppm PBB; (×) 200 ppm PBBs. Each point represents the mean for 3–12 mice. Standard errors (not shown for clarity) were approximately 10% of mean values. The asterisks (*) indicate plasma ICG concentration significantly different from 0 ppm PBBs ($p < 0.05$). From Cagen et al. (*34*).

Table 3. Initial rate of elimination of ICG from plasma in 21-day-old rats exposed to dietary PBBs.

PBBs, ppm[a]	Rate of elimination (slope)[b]	(±) Fiducial limits	$T_{\frac{1}{2}}$, min
0	−0.025	0.010	10.5
50	−0.042[c]	0.013	7.0

[a] Concentration of PBBs in mother's diet from day of birth until day of experiment.
[b] ICG (40 mg/kg) injected via the tail vein and rate of elimination of ICG from plasma determined from plasma ICG concentrations at 1, 5, 10, and 15 min following injection by the method of least squares.
[c] Significantly different from 0 ppm PBBs ($p < 0.05$).

uptake in 21-day-old rats was not associated with enhanced ouabain excretion (Table 2) and was associated with significantly lower plasma ouabain concentration at only the earliest (3 min) time interval (Fig. 1). Two week dietary exposure to PBBs in adult mice resulted in enhanced initial disappearance of ICG from plasma (Fig. 4), which provides

Table 4. Elimination of sulfobromophthalein (BSP) from plasma and biliary excretion of BSP following PBBs in adult rats.

PBBs, ppm[a]	Parameter	Time following BSP administration[b] 3 min	10 min	20 min	30 min	40 min
0	BSP remaining in plasma, mg/100 ml	157.8 ± 1.8	75.8 ± 1.4	48.5 ± 1.9	39.4 ± 1.4	33.0 ± 1.9
100	BSP remaining in plasma, mg/100 ml	131.8 ± 6.1[c]	61.8 ± 2.7[c]	33.2 ± 2.4[c]	18.9 ± 2.6[c]	7.3 ± 2.0[c]
0	BSP excretion, μmole/min/kg	—	0.16 ± 0.06	0.78 ± 0.15	0.83 ± 0.13	0.96 ± 0.04
100	BSP excretion, μmole/min/kg	—	0.71 ± 0.06[c]	1.86 ± 0.09[c]	2.27 ± 0.24[c]	2.22 ± 0.11[c]
0	Metabolites of BSP in bile, %	—	24 ± 16	64 ± 8	63 ± 8	76 ± 3
100	Metabolites of BSP in bile, %	—	74 ± 4[c]	89 ± 3[c]	92 ± 2[c]	96 ± 1[c]

[a] Concentration of PBBs in diet of adult rats for 2 weeks.
[b] BSP (120 mg/kg) was injected into the femoral vein of anesthetized rats and at various times following BSP administration, the amount of BSP in plasma and bile was determined.
[c] Significantly different from 0 ppm PBBs ($p < 0.05$).

additional evidence for enhanced uptake capacity following exposure to PBBs.

It is difficult to determine to what extent enhanced uptake capacity is due to specific stimulation of transport mechanisms or increased liver mass. Stimulation of drug transport following phenobarbital has been attributed, by some, to be due to increased hepatic blood flow resulting from a greater liver mass (*24–26*). A similar relationship may exist following PBBs.

In adult rats, Meijer et al. (*27*) suggested that the rate-limiting step for ouabain excretion from blood into bile is biliary excretion (not hepatic uptake). Thus, stimulation of uptake mechanisms in adult mice and rats following PBBs might not be expected to enhance overall excretion of ouabain into bile. Newborn rats (*28, 29*) and possibly newborn mice (*20*) are immature in their ability to take up compounds into liver, and thus, stimulation of uptake following PBBs may be more important for overall transport in these animals (Table 2).

Exposure of adult rats to 100 ppm PBBs resulted in enhanced plasma disappearance and biliary excretion (Table 4) of BSP. Moreover, treated rats excreted a greater percentage metabolized BSP than controls (Table 4). Whelan et al. (*30*) suggested that BSP metabolism (conjugation to glutathione) is rate-limiting in BSP transport from blood into bile. Since exposure to PBBs resulted in increased biliary excretion of BSP metabolites (Table 4) and *in vitro* conjugation of BSP is stimulated in rats treated with PBBs (*31*), the effect of PBBs on BSP transport may be primarily attributed to increased metabolism.

The results of this investigation therefore demonstrate that PBBs have the capacity to alter the hepatic drug elimination processes, but the magnitude of the effect is dependent on age and transported compound. Since many drugs and environmental chemicals are excreted by the liver (*32, 33*), the potential exists for chemical interactions following exposure to PBBs. It is noteworthy that 15-day-old rats whose mothers were exposed to 50 and 100 ppm PBBs were protected against ouabain-induced lethality (*20*).

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